

WE CLAIM:

1. A method for producing beta-carotene and carotene-related pigments comprising the steps of:

collecting a plurality of thermophilic microorganisms and screening said thermophilic microorganisms for the production of pigments;

identifying and separating at least one pigment-producing thermophilic microorganism from said plurality of thermophilic microorganisms, said at least one pigment-producing thermophilic microorganism producing pigments having at least one of yellow, red and orange coloration; and

mutating by one of recombinant and non-recombinant means said at least one pigment-producing thermophilic microorganism to enhance pigment production, forming a mutant pigment-producing thermophilic microorganism.

2. A method in accordance with Claim 1, wherein said at least one pigment-producing microorganism is mutated by spreading at least one cell solution comprising said at least one pigment-producing thermophilic microorganism onto TT medium agar plates comprising nitrosguanidine (NTG) crystals and incubating said plates at an elevated temperature, resulting in formation of mutant colonies proximate said NTG crystals.

3. A method in accordance with Claim 2, wherein at least one mutant colony suitable for over-producing carotene is separated from said mutant colonies.

4. A method in accordance with Claim 2, wherein said plates are incubated at a temperature of at least about 52°C.

5. A method in accordance with Claim 1, wherein said mutant pigment-producing thermophilic microorganism is GTI-CARD.

6. A method in accordance with Claim 1 further comprising introducing a gene of interest suitable for producing a protein of interest into said mutant pigment-producing thermophilic microorganism, resulting in over-production of said carotene pigment and said protein of interest.

7. A method in accordance with Claim 6, wherein said gene of interest is introduced into said mutant pigment-producing thermophilic microorganism using a DNA molecule comprising maintenance means for maintaining at least one of plasmids and integrative vectors in a *Thermus* host and expression means for expressing at least one of homologous genes and heterologous genes.

8. A method in accordance with Claim 7, wherein said plasmids comprise a *Thermus* promoter sequence adjacent to an insertion site for insertion of DNA fragments.

9. A method in accordance with Claim 7, wherein said expression means comprises a 5' untranslated region added to a 5' end of a transcript whereby gene expression and mRNA stability/longevity increase.

10. A method in accordance with Claim 7, wherein said expression means comprises a ribosomal binding site addition to an expression vector.

11. A method in accordance with Claim 7, wherein said expression means comprises at least one inducible promoter.

12. A method in accordance with Claim 7, wherein said expression means comprises at least one multiple cloning site.

13. A method in accordance with Claim 7, wherein said expression means comprises a *Thermus* transcriptional termination sequence flanking said gene of interest and its associated promoter.

14. A method in accordance with Claim 7, wherein said DNA molecule comprises a *Thermus* transcriptional termination sequence flanking a gene of interest and its associated promoter, a 5' untranslated region added to a 5' end of a transcript whereby gene expression and DNA molecule sequences increase, at least one multiple cloning site, a ribosomal binding site addition to an expression vector and at least one inducible promoter.

15. A *Thermus* culture comprising:
means for over-producing at least one carotene, said means comprising a mutation in a biosynthesis pathway suitable for over-producing carotene.

16. A *Thermus* culture in accordance with Claim 15, wherein said at least one carotene is beta-carotene.

17. A *Thermus* culture in accordance with Claim 15 further comprising at least one DNA molecule suitable for expressing at least one of heterologous proteins and homologous proteins in a *Thermus* host also suitable for over-producing said carotene.

18. A *Thermus* culture in accordance with Claim 17, wherein said DNA molecule comprises maintenance means for maintaining at least one of plasmids and integrative vectors in said *Thermus* host and expression means for expressing at least one of heterologous genes and homologous genes.

19. A *Thermus* culture in accordance with Claim 17, wherein said DNA molecule comprises a *Thermus* promoter sequence adjacent to an insertion site for insertion of DNA fragments.

20. A *Thermus* culture in accordance with Claim 17, wherein said expression means comprises a 5' untranslated region added to a 5' end of a transcript whereby gene expression and mRNA stability/longevity increase.

21. A *Thermus* culture in accordance with Claim 17, wherein said expression means comprises at least one multiple cloning site.

22. A *Thermus* culture in accordance with Claim 17, wherein said expression means comprises a ribosomal binding site addition to an expression vector.

23. A *Thermus* culture in accordance with Claim 17, wherein said expression means comprises at least one inducible promoter.

24. A *Thermus* culture in accordance with Claim 17, wherein said expression means comprises a *Thermus* transcriptional termination sequence flanking a gene of interest and its associated promoter.

25. A DNA molecule comprising:
maintenance means for maintaining at least one of plasmids and integrative vectors in a *Thermus* host and expression means for expressing at least one of homologous genes and heterologous genes.

26. A DNA molecule in accordance with Claim 25, wherein said plasmids comprise a *Thermus* promoter sequence adjacent to an insertion site for insertion of DNA fragments.

27. A DNA molecule in accordance with Claim 25, wherein said expression means comprises a 5' untranslated region added to a 5' end of a transcript whereby gene expression and mRNA stability/longevity increase.

28. A DNA molecule in accordance with Claim 25, wherein said expression means comprises at least one multiple cloning site.

29. A DNA molecule in accordance with Claim 25, wherein said

expression means comprises a ribosomal binding site addition to an expression vector.

30. A DNA molecule in accordance with Claim 25, wherein said expression means comprises at least one inducible promoter.

31. A DNA molecule in accordance with Claim 25, wherein said expression means comprises a *Thermus* transcriptional termination sequence flanking a gene of interest and its associated promoter.

32. A DNA molecule in accordance with Claim 25 further comprising a *Thermus* transcriptional terminator sequence flanking a gene of interest and its associated promoter, a 5' untranslated region added to a 5' end of a transcript whereby gene expression and mRNA stability/longevity increase, at least one multiple cloning site, a ribosomal binding site addition to an expression vector and at least one inducible promoter.